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Infrared Identification of Disaccharides

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▶ **The value of infrared spectra for the identification of amorphous disaccharides and their acetates, by comparison with spectra of known disaccharides and their acetates, is demonstrated. Infrared spectra of ten amorphous disaccharides of D-glucose, of D-glucose and D-fructose, and of their β-octaacetates are presented over the range of 650 to 1500 cm.$^{-1}$ Potassium bromide disks were used. All spectra differ in sufficient detail to allow differentiation among closely related disaccharides.**

Although chromatographic analysis has been of inestimable value in separation of mixtures of sugars, their identification by chromatographic evidence alone is not conclusive. Relative rates of migration on paper or column, together with color reactions with spray reagents, only contribute to identification. Physical properties of isolated samples or derivatives are also required. Heretofore, crystallization of the sugar or derivative has been considered imperative before an identification is unequivocal.

Sugar samples isolated by chromatographic means may be so small that it is practically impossible to crystallize them. Hence, melting point, x-ray diffraction, and crystallographic study are eliminated as tools for identification. Determination of optical rotation does not require crystalline samples, but values may become only approximations as sample weights decline into the lower milligram range.

The infrared absorption spectrum of a compound is increasingly used for its identification and analysis. As a unique physical property that is not primarily dependent on crystallization, it offers a most useful criterion for identification of disaccharides.

Infrared spectra of all types of carbohydrates have been published. In the first of a series of papers on this subject, Barker and his colleagues (*5*) stated that determinations of infrared spectra offer powerful means for the comparison of supposedly identical samples of carbohydrates. They examined the spectra of a variety of carbohydrates and their derivatives, but have not published them in sufficient detail for comparison purposes. They noted that spectra in the 730- to 960-cm.$^{-1}$ range allow assignment to the α- or β-series of D-glucopyranoses. Whistler and House (*20*) have also reported that certain regions of absorption are characteristic of the configuration of the anomeric carbon.

Kuhn (*13*) has published the spectra of 79 carbohydrates and derivatives over the range 8.0 to 15.0 microns. Of these, only two are of sugars included here. Kuhn's spectra were determined with an amount of sample in the beam too small to permit maximum utility of the curves for comparison purposes.

Infrared spectra of a large number of sugar acetates and related compounds have been determined at the National Bureau of Standards (*12*). Crystalline materials were used but the spectra were all determined in solution.

The pressed-disk technique is useful when water-soluble materials are examined. It also permits the use, where necessary, of less-than-milligram samples (*1*). Some difficulties have been experienced in comparing disk spectra with those obtained from mulls (*2, 3, 8*). Barker *et al.* (*6*) have ascribed the changes they previously (*3*) noted during aging of disks containing certain carbohydrates to the relatively high moisture content of potassium bromide disks. They did not find any such changes in any of the three disaccharides included in their study. The value of infrared spectra in identification of larger molecules such as trisaccharides was cited by Whiffen (*19*), who noted that identification by melting point and rotation

becomes rather uncertain at this level of complexity.

With potassium bromide disks and a standardized procedure for converting the sugar to the acetate, infrared spectra may be obtained of quality sufficient to allow identification with 10 mg. of amorphous sugar, without the use of supplementary condensing systems in the spectrophotometer. In these spectra of the free sugar and the acetate, as many as 20 to 30 items of information are available from one sugar for comparison with an unknown, contrasted with one for a melting point.

Degree of crystallinity has a considerable influence on solid-state infrared spectra. The spectra reported here are those of noncrystalline sugars and acetates; the procedure adopted is intended to avoid introducing crystallinity as a variable. The spectra of these amorphous materials do not show as much detailed structure as those of crystalline substances; the number and intensities of bands are considerably reduced. This numerical reduction of bands is compensated for by including the spectrum of the acetate in the comparison.

APPARATUS AND REAGENTS

Spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer. The resolution dial was set at 930, no reference was used in the rear beam, and the optical balance was turned maximum clockwise. (Preliminary experiments indicated that although disks of very good optical quality could be pressed with freeze-dried sugars in potassium bromide, disks containing the sugar concentration required could not be removed from the die without cracking.) A die that could be inserted into the spectrophotometer without removal of the disk was made by modifying commercial punches and dies designed for punching sheet metal. The punch was 0.500 inch in diameter with a straight shank. The dies were 1.00 inch in outside diameter and 1.187 inches long. The internal diameter, originally 0.497 inch, was enlarged to 0.5005 inch. The vacuum chamber was similar to that of the press distributed by Jarrel-Ash Co. (Catalog No. H920) with the addition of screws for applying force to remove the punch from die after pressing. These dies fit the microcell adapter of the spectrophotometer, except that the two shelves for positioning the microcell interfere. A new adapter was made having removable shelves. With this arrangement, the die containing the potassium bromide disk could be inserted directly into the microcell adapter for obtaining the spectrum.

Lemieux *et al.* (*14*) and Grendon and Lovell (*10*) have also designed dies which could be inserted directly into a Model 21 spectrophotometer.

Disaccharide Samples. Sucrose, maltose, turanose, cellobiose, and gentiobiose were obtained commercially.

Isomaltose was obtained from enzymatic hydrolysis of NRRL B-512 dextran.

Leucrose, sample NRRL 3674-50-E.

Maltulose was prepared by isomerization of maltose by limewater at 35° C. for 3 days under toluene. After excess maltose was removed by digestion with honey invertase (*22*), the maltulose was separated by charcoal column and paper chromatography. From 1 gram of maltose was obtained 103 mg. of amorphous maltulose, $[\alpha]_D^{25} = +52°$ [+52.8° (*18*), +56.2° (*7*)]. The reducing power of the sugar was 41.7% of that of glucose (maltose was 43.9%) with Shaffer-Somogyi reagent 50. The sugar consumed 91% less iodine in the hypoiodite oxidation than did maltose, showing the reducing group to be ketose. Its osazone showed an identical x-ray powder diffraction pattern to that of maltosazone.

Inulobiose. A disaccharide fraction was prepared by the partial hydrolysis of inulin as directed by Pazur and Gordon (*17*).

Nigerose. Five preparations of this sugar were as follows:

1. By acid reversion from D-glucose at The University of Nebraska.
2. By acid reversion essentially as described by Pazur and Budovich (*16*), $[\alpha]_D^{25} = +86°$; Pazur and Budovich reported +87°.
3. From sakébiose octaacetate. Matsuda *et al.* showed sakébiose to be identical with nigerose (*15*). A sample of crystalline sakébiose acetate (30 mg.) was treated in methanol (2 ml.) with 0.1 ml. of 0.4*N* barium methylate (*11*) at 5° C. for 24 hours. The suspension was treated with 0.4 ml. of 0.1*N* sulfuric acid and centrifuged, and the supernatant was evaporated to dryness, dissolved in water, and deionized by ion exchange.
4. By hydrolysis of nigeran. Nigeran was prepared from *Aspergillus niger* mycelia as described by Yuill (*24*). It was subjected to partial acid hydrolysis followed by destruction of maltose by baker's yeast. A disaccharide was obtained by charcoal column chromatography corresponding to that obtained by Barker *et al.* (*4*) from nigeran hydrolysis. Yields were too low to permit reliable optical rotation determination.
5. By isolation from honey. A reducing disaccharide was isolated from honey by carbon column and paper chromatographic procedures (*21*). It was hydrolyzed to D-glucose only and it migrated on paper slightly faster than maltose.

PROCEDURE

Sugars. To a solution of the sugar (3 mg. in 10 ml. of water) is added 500 mg. of potassium bromide (Harshaw infrared quality). A second solution (10 ml.) is prepared at 0.25 of the concentration of the first and 500 mg. of potassium bromide is added. Both solutions are freeze-dried and the dry products are transferred to a mullite mortar and lightly ground. A 400-mg. sample is weighed, dried for 3 hours in vacuo at 85° C., and stored (as are disks) in a desiccator. The spectra of the disks are determined immediately after pressing. The disks (diameter, 1.26 cm.; thickness, approximately 0.1 cm.) are pressed for 1 minutes at a total force of 10 tons (50 tons per square inch).

Sugar Acetates. The acetates are prepared by the procedure described by French (*9*), which has been found satisfactory for routine preparation of milligram quantities of completely acetylated disaccharides for infrared spectra. The sugar (5 to 10 mg.) is mixed with an equal weight of fused sodium acetate and 0.05 to 0.10 ml. of acetic anhydride in a 10 × 75 mm. test tube. The suspension is boiled by touching the test tube to a hot plate until solution occurs (about 1.5 minutes). The sample is spread over the lower half of the inside of the test tube by rotating it as it cools and solidifies. A stream of warm air is directed into the test tube until the residual acetic anhydride is evaporated (about 4 to 6 hours). The residue is extracted several times with a few milliliters of benzene which is filtered through sintered glass and evaporated dry on the steam bath. Yields are 70 to 80% of theory.

The octaacetates are deposited from acetone solution onto powdered (200-mesh) potassium bromide. To 410 mg. of potassium bromide in a mullite mortar is added the sugar acetate (3.2 mg. in 0.10 to 0.15 ml. of acetone). The entire volume should be taken up by the dry powder. It is mixed in the mortar by gentle rubbing until after the solvent evaporates. A diluted sample is made up similarly using 0.5 mg. of acetate and 410 mg. of potassium bromide.

An alternative procedure for preparation of the dilute samples is to powder the original disks after use and mix a portion with the required amount of potassium bromide. Reduction of disk thickness by pressing one fourth of the material at the original concentration is not satisfactory for routine work, as the strength of the disks is too low at the reduced thickness.

RESULTS AND DISCUSSION

The infrared spectra of these disaccharides and their octaacetates are shown in Figures 1 and 2. Any one of these carbohydrates may be distinguished from the remainder by comparison of spectra. The range included is from 650 to 1500 cm.$^{-1}$, as the spectra at higher frequencies are of no value for this purpose. Concentrations are: sugars, 1.5 and 6.0 mg. per gram of potassium bromide; acetates, 1.5 mg. and 8.0 to 9.0 mg. per gram of potassium bromide.

Effect of Crystallinity. In Figure 3 are shown two spectra of sucrose. The upper curve is that of crystalline sucrose, mixed with 200-mesh potassium bromide and dried at 100° C. in vacuo for 1 hour before pressing.

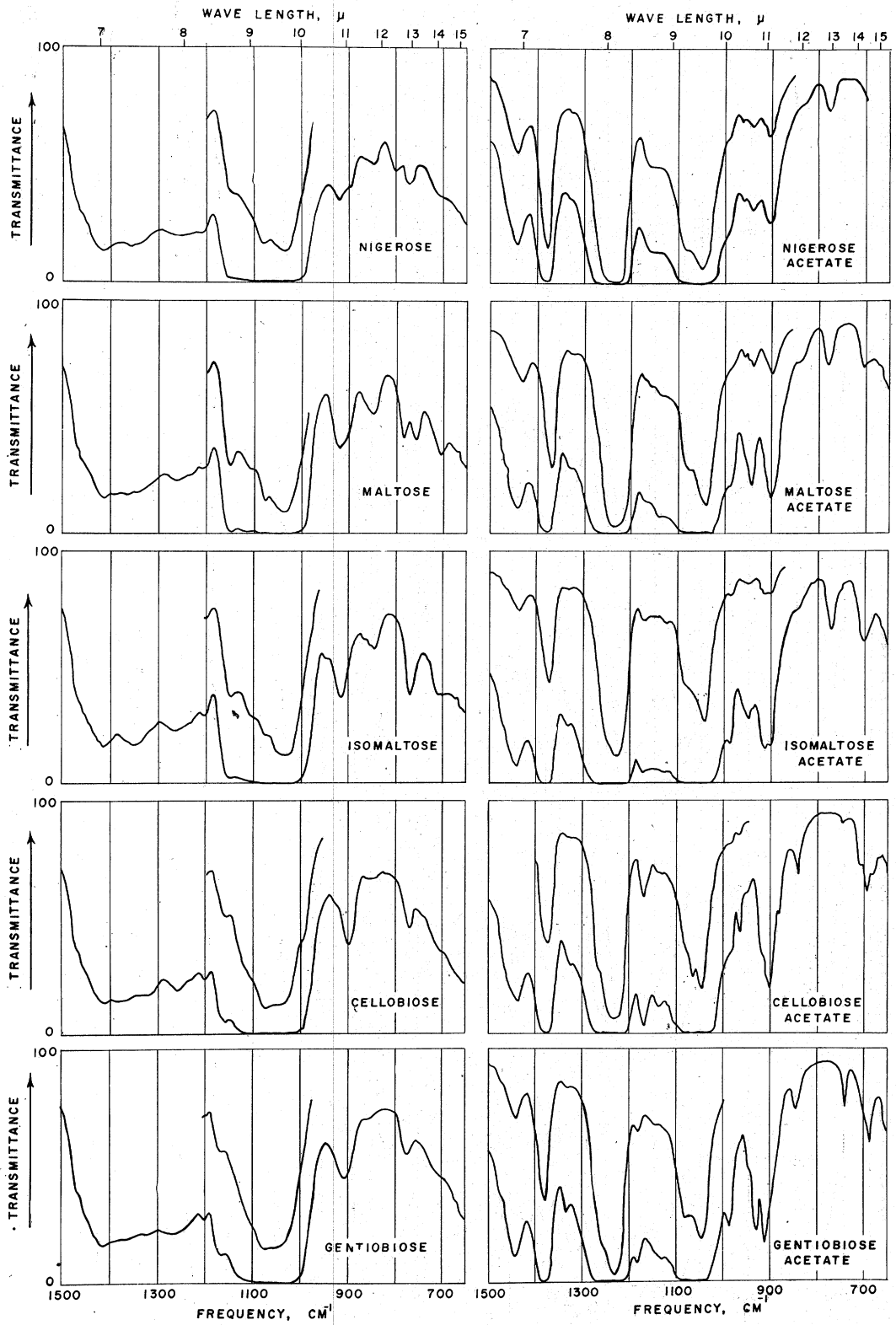

Figure 1. Infrared spectra of various disaccharides and their octaacetates in potassium bromide disks, 1.1 to 1.2 mm. thick

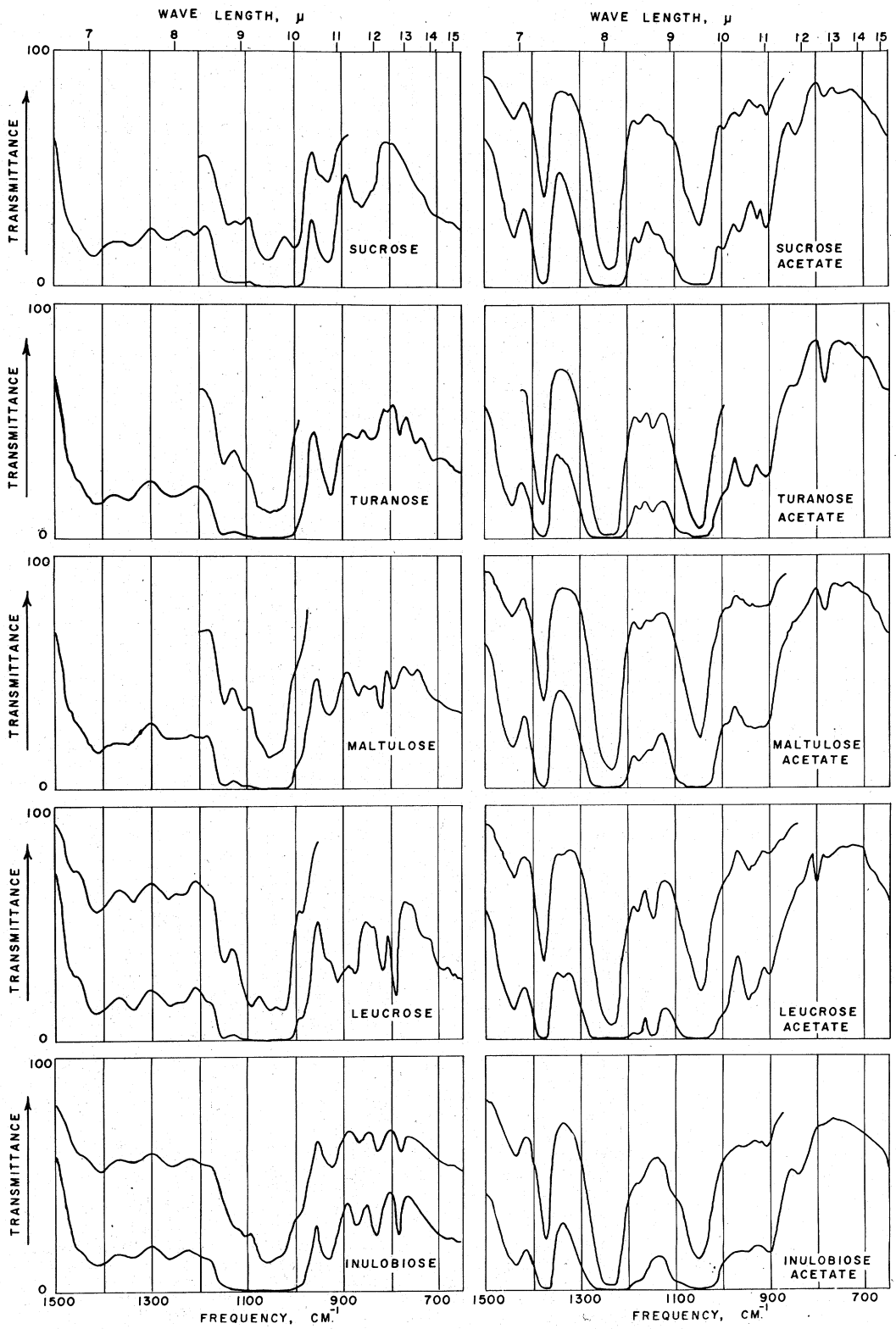

Figure 2. Infrared spectra of various disaccharides and their octaacetates in potassium bromide disks, 1.1 to 1.2 mm. thick

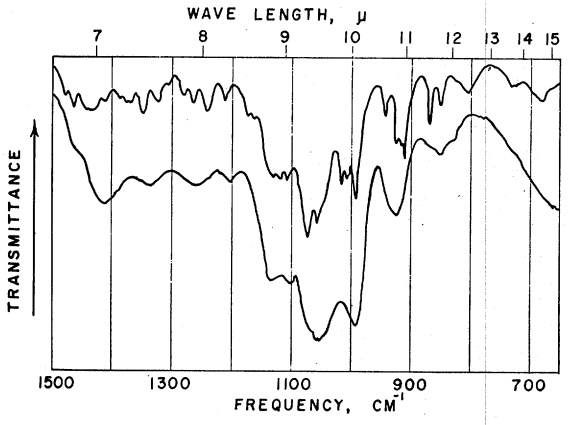

Figure 3. Infrared spectra of sucrose

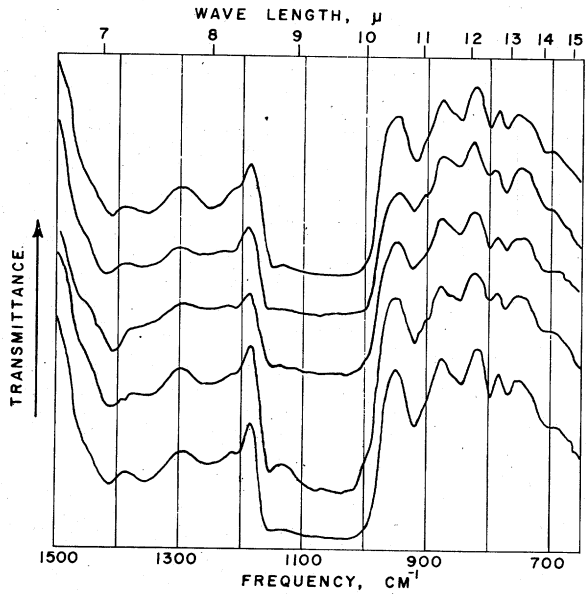

Figure 4. Infrared spectra of five preparations of 3-O-α-D-glucopyranosyl-D-glucose

Considerably greater detail is shown than in the lower curve, which is that of a freeze-dried sucrose–potassium bromide solution prepared as described above, but not heated after drying. The upper curve shows detail which is the same or better than that of a spectrum of crystalline sucrose from a mineral oil mull. The curves have been vertically displaced to facilitate comparison.

To investigate the possibility that the differences in spectra are due to formation of low-temperature sucrose hydrates in the freeze drying, samples of sucrose–potassium bromide mixtures dried in this way were heated in a vacuum oven. Young and Jones (*23*) have reported that two sucrose hydrates formed at freezing temperatures: $C_{12}H_{22}O_{11}.2.5\ H_2O$, melting point 45.7° C.; and $C_{12}H_{22}O_{11}.3.5\ H_2O$, melting point 27.8° C. Both decomposed to anhydrous sucrose at their melting points. Heating of the dry sucrose–potassium bromide mixture for 4 hours at 110° C. in a vacuum of 0.7 mm. of mercury produced no change in the spectrum, except a slight lowering of the water band at 1630 cm.$^{-1}$

Reproducibility. Figure 4 shows the spectra of the five preparations of 3-*O*-α-D-glucopyranosyl-D-glucose. The curves have been vertically displaced to facilitate comparison. From top to bottom the curves show preparation from sakéboise, nigerose by acid reversion (this laboratory), nigerose by acid reversion (University of Nebraska), nigerose from nigeran, and nigerose from honey. All maxima fall within ±5 cm.$^{-1}$ of the mean value for each band. Four replicate preparations were made of sucrose octaacetate by the procedure outlined above. Their spectra were superimposable.

Four of the sugar acetate spectra shown here were also published by Isbell and coworkers (*12*). Their spectra were determined on solutions of recrystallized materials. The spectra resemble those shown in this paper closely, except that minor peaks are more difficult to compare at the lower concentrations used by Isbell—e.g., the spectrum of gentiobiose octaacetate shown here differs in showing more structure in the 650- to 1000-cm.$^{-1}$ region. Thus the infrared spectra of amorphous acetates deposited on potassium bromide resemble solution spectra rather than solid-phase spectra of crystalline materials.

The use of the procedure outlined here for the identification of the disaccharides of honey will be described in a forthcoming publication.

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